JOURNAL OF BACTERIOLOGY, Nov. 1982, p. 919–923
0021-9193/82/110919-05$02.00/0

Vol. 152, No. 2

Escherichia coli Polypeptide Controlled by the *lon* (*capR*) ATP Hydrolysis-Dependent Protease and Possibly Involved in Cell Division

J. M. SCHOEMAKER,* G. W. HENDERSON, AND A. MARKOVITZ

Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

Received 28 December 1981/Accepted 27 July 1982

Mutation in the gene *lon* (*capR*) of *Escherichia coli* K-12 causes conditional inhibition of cell division. Two-dimensional gel electrophoresis was used to compare polypeptides from isogenic *capR*$^+$ and *capR* strains. One polypeptide was present in the *capR* strain but absent in the wild-type strain, and it was proteolyzed when the pure *capR*$^+$ protease was added to the *capR* extract. This polypeptide could only be detected in the *capR* strain when cell division was inhibited, and its synthesis was independent of the SOS response.

The *lon* (*capR*) gene product of *Escherichia coli* K-12 has been identified as a 94-kilodalton polypeptide (29, 33, 34) that has an ATP-hydrolysis-dependent protease activity, an ATPase activity (7), and an affinity for DNA (33). *capR* mutants that are defective in this multi-activity enzyme display several phenotypic alterations. These include the overproduction of capsular polysaccharide (24), increased sensitivity to UV and ionizing radiation (1, 9, 18, 24), inhibition of cell division (1, 18), failure to lysogenize the λ (11, 32) and P1 phages (31), and reduced degradation of abnormal (6, 15, 20, 30) and normal (10, 14) polypeptides. Two possible ways for the *capR* protein to function in the varied cellular pathways associated with the above-mentioned phenotypes are (i) by proteolytically activating or inactivating substrates and (ii) by regulating the transcription of operons, for example, as a classical repressor.

Evidence for the second mode of action of the *capR* protein comes from studies on the regulation of capsular polysaccharide synthesis. Of the enzymes involved in this biosynthesis, 10 are elevated in *capR* strains (24). For the most studied of these operons, the galactose operon, increased levels of mRNA have been detected in *capR* mutants (5, 23), contributing evidence for a second operator in the operon that responds to *capR* control (24). Evidence for *capR*$^+$ protein acting in a proteolytic fashion on native proteins has so far been indirect. The functional and chemical half-life of the λ *N* gene product is increased several-fold in *capR* cells (14). Also, the precursor of *E. coli* K-12 major outer membrane protein *a* appears to be more stable in a *capR* mutant (10), as does the *sulA* protein (S. Mizusawa and S. Gottesman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, H130, p. 134). However, the exact nature of the interaction of the *capR*$^+$ protein with these polypeptides has not yet been determined.

In the present study, two-dimensional gel electrophoresis was used to identify proteins which are affected by the *capR*$^+$ protein. In particular, specific targets of the *capR*$^+$ proteolytic activity were sought by treating cell extracts in vitro with the purified *capR*$^+$ protease before electrophoresis. One of the phenotypes associated with a mutation in the *capR* gene is inhibited cell division. This phenomenon has been observed after treatment with UV or ionizing radiation followed by plating on complex media (1, 18) and after transfer of the *capR* strain from minimal to complex media in the absence of radiation (3, 12). In this study, conditions in which the cells stopped dividing normally were selected to look for a protein(s) which is associated with this phenomenon and which may be a target for *capR*$^+$ regulation. The *capR* wild-type strain, X7102 (*proC trp* Δ*lac X74 str* containing no known suppressors), and the isogenic spontaneous *capR* strain, X7102-82 (containing the *capR* allele *capR82* [29]) were grown at 37°C to exponential phase in glucose minimal medium (12) and then were diluted into double strength Bacto methionine assay medium (complex medium), a condition which caused the cells to cease actively dividing but did not significantly reduce [^{35}S]methionine incorporation. After a 60-min incubation, the cells were labeled for 30 min with L-[^{35}S]methionine (0.015 mCi/ml, 998.9 Ci/mmol; New England Nuclear Corp.). Cultures of 10 ml were harvested, washed three times in buffer (10 mM Tris-hydrochloride [pH 8.0], 1 mM EDTA, 1 mM 2-mercaptoethanol,

100 mM NaCl as modified [16]), and suspended in 300 μl of the same buffer for disruption in a sonic oscillator. The suspension of broken cells was brought to 10% glycerol–10 mM $MgCl_2$–10 mM $CaCl_2$–400 ng of DNase 1 per ml–2 μg of micrococcal nuclease (Sigma Chemical Co.) per ml and was incubated for 3 min at 37°C (16).

The broken-cell suspensions that were treated in vitro with the *capR*$^+$ ATP-dependent protease were first centrifuged at 12,000 × *g* for 20 min to remove the cell envelope fraction. Protein portions of 10 μg from the supernatant fractions were treated with purified *capR*$^+$ protein in a standard protease assay containing 50 mM Tris-hydrochloride (pH 8.0)–10 mM $MgCl_2$–2 mM ATP–2.2 μg of homogeneous *capR*$^+$ protein (7) in a total volume of 40 μl. Reaction mixtures were incubated at 37°C for 90 min, and the reactions were terminated by freezing the extracts. Control assays run in parallel contained the same ingredients except for *capR*$^+$ protein. Each preparation was electrophoresed at least in duplicate as previously described (4, 25–27).

Differences in the concentration or location of several polypeptides were found when the two-dimensional gels of the labeled proteins of the wild-type and *capR* strains were compared (Fig. 1). *capR*$^+$ protein itself is visible in the wild-type strain (Fig. 1, spots labeled b) but not in the mutant. The *capR*$^+$ protein probably has multiple isoelectric forms since the two spots also appear substantially larger in gels prepared from the wild-type strain containing a multicopy *capR*$^+$ plasmid, pJMC40 (29; data not shown). In addition, the pure *capR*$^+$ protein (7) shows four isoelectric forms on two-dimensional gels (T. A. Phillips and F. C. Neidhardt, personal communication).

Remarkably, only one polypeptide was detected that appeared to be a substrate of *capR*$^+$ ATP-dependent proteolytic activity. It appeared to be a substrate for two reasons. First, it appeared at a high concentration in gels of the *capR* strain but was greatly reduced in gels of the wild-type strain (Fig. 1, protein labeled a). Second, it appeared much less concentrated in those gels prepared from *capR* extracts that were treated in vitro with *capR*$^+$ protease (Fig. 2). Adding the protease inhibitor phenylmethylsulfonyl fluoride during preparation of the cell extracts affected neither the appearance of polypeptide *a* in the *capR* strain nor its absence in the wild-type strain. Also, polypeptide *a* was present at a high concentration in the *capR* strain after only a 10-min labeling as well as after the 30-min labeling shown in Fig. 1.

Polypeptide *a* is the first bacterial protein shown to be a target for regulation by the *capR*$^+$ proteolytic activity. It may be either a direct substrate of the *capR*$^+$ protease or part of a cascade initiated by *capR*$^+$ proteolysis. Since it was not in a purified form in the protease assay, it is still possible that the *capR*$^+$ protease was

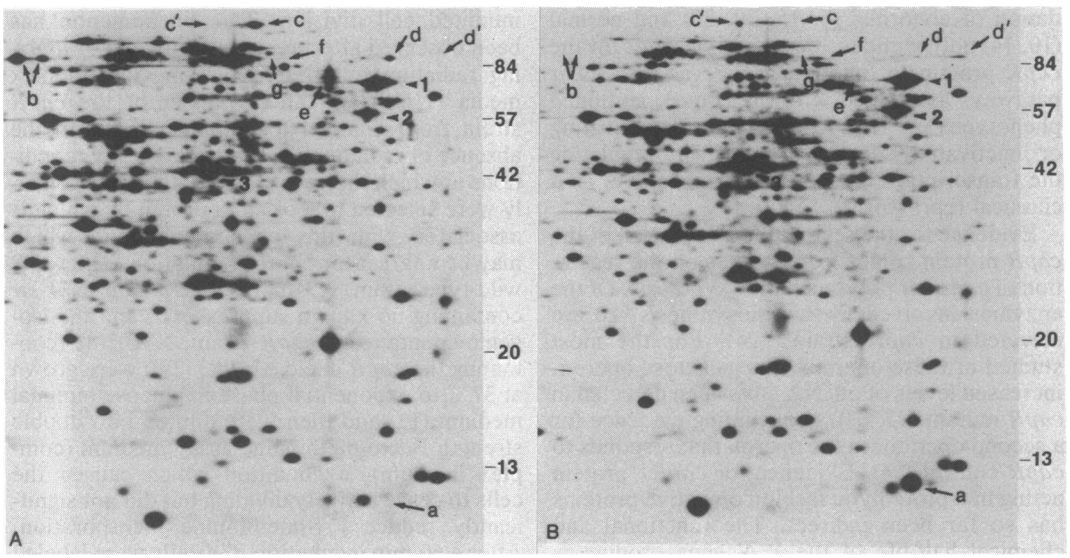

FIG. 1. Two-dimensional gels of [^{35}S]methionine-labeled polypeptides from (A) the *capR* wild-type strain X7102 and (B) the *capR* mutant strain X7102-82. From left to right, the gels show proteins of decreasing isoelectric points. The approximate molecular weights (× 10^3 scale) of the second dimension as well as the locations of marker proteins were determined by comparison of these gels with those of Bloch et al. (4). Marker proteins are (1) ribosomal protein S1, (2) *groE* gene product, and (3) elongation factor Tu.

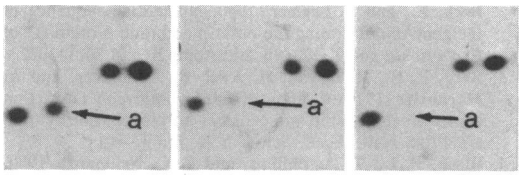

FIG. 2. Two-dimensional gels comparing the concentrations of polypeptide *a* after incubation of cell extracts with or without the *capR*$^+$ ATP-dependent protease. Photos correspond to the lower right-hand sections of the gels in Fig. 1. Left: Strain X7102-82, no *capR*$^+$ protease treatment. Center: Strain X7102-82 treated with *capR*$^+$ protease. Right: Strain X7102, no *capR*$^+$ protease treatment.

acting indirectly by, for example, activating some other protease in the cell extracts which then proteolyzed polypeptide *a*.

Given the phenotypes associated with a mutation in the *capR*$^+$ gene, the polypeptide *a* could be (i) an enzyme of capsular polysaccharide synthesis or an activator of such enzymes, or (ii) a protein involved in inhibiting cell division or a precursor of a protein needed for cell division. Polypeptide *a* did not appear in the *capR* strain when grown under conditions which maximize capsular polysaccharide synthesis (growth in minimal medium). This strongly suggests that this polypeptide is not an enzyme involved in capsular polysaccharide synthesis. It appeared only in the *capR* strain after the shift to a complex medium, a condition in which the cells essentially stopped dividing. Therefore, polypeptide *a* could be a protein involved in inhibiting cell division.

A current model describing the role of the *capR*$^+$ protein in inhibiting cell division relates to the SOS pathway. After damage to the chromosome, the *recA* protein is activated proteolytically, resulting in cleavage of the *lexA* protein and increased expression of the proteins under *lexA* protein control, including the *sulA* protein (8, 13, 19, 22, 28). The latter is thought to be responsible for the subsequent inhibition of cell division which is transient in *capR* wild-type cells but permanent in *capR* cells after SOS induction. Thus, the *capR*$^+$ protein is thought to be involved in the SOS response by regulating the *sulA* protein, perhaps through proteolysis (19). This is supported by the recent observation that the *sulA* protein is more stable in a *capR* strain (S. Mizusawa and S. Gottesman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, H130, p. 134).

Based on the molecular weight of polypeptide *a* (approximately 11,000; Fig. 1), it seemed unlikely to be the *sulA* protein, whose molecular weight is approximately 18,000 (Mizusawa and Gottesman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, H130, p. 134; Fig. 1). However, the possibility remained that polypeptide *a* is another protein necessary for inhibiting cell division and is induced as part of an SOS response resulting from growth in complex media. This possibility was tested in two experiments. (i) Cell extracts of a *capR* strain labeled in complex medium were compared with extracts of an identically grown isogenic strain carrying a missense mutation in the *recA* gene (*recA1*), which produces a functionally inactive *recA* protein (17). Polypeptide *a* was present at a high concentration in the *recA1* mutant, indicating that its expression does not require functional *recA* protein and an SOS response. (ii) An SOS response was induced in a *capR* strain growing in minimal medium by incubating for 30 min with nalidixic acid. Polypeptide *a* was not detected in two-dimensional gels, although the induction of other proteins, including the *recA* protein, was observed. This suggests that polypeptide *a* is not part of the SOS response. However, the possibility remains that it is part of another pathway, as yet unknown, leading to the inhibition of cell division.

In addition to polypeptide *a*, there are several other obvious polypeptide changes between the wild-type and *capR* strains shown in Fig. 1. Two of the polypeptides, for example, appear to be shifted in the isoelectric focusing dimension between the mutant and wild-type strains (compare c, c′ and d, d′). Other polypeptides appear to be at high concentrations in one strain (polypeptides *e* and *g* in the wild-type strain, polypeptide *f* in the mutant) and missing or at low concentrations in the other. Two of the changed polypeptides have been identified by comparing their locations in two-dimensional gels with those published elsewhere (4, 27). Polypeptide *c* is the β subunit of RNA polymerase, and polypeptide *e* is dihydrolipoamide acetyltransferase (encoded by gene *aceF*), an enzyme of the pyruvate dehydrogenase complex (21). Surprisingly, all these polypeptide differences are due to the presence of capsular polysaccharide in cell extracts of the *capR* strain. This conclusion was drawn from the following experiments. Labeled extract of the wild-type strain was mixed with either unlabeled extract from the *capR* strain (ratio of wild-type to *capR* strain protein, 1:3) or with purified capsule (4 μg of protein to 1 μg of polysaccharide) before electrophoresis. The two-dimensional gels of these treated wild-type extracts appeared identical to gels of untreated *capR* extracts with respect to the appearance of the polypeptides mentioned above (except for polypeptide *a*). In addition, two-dimensional gels of an unencapsulated *capR* strain carrying a mutation in the *galU* gene, which results in the inhibition of capsular poly-

saccharide synthesis (24), were indistinguishable from the isogenic wild-type strain with regard to these polypeptides. Polypeptide *a*, however, was still present at a high concentration in the *capR galU* strain.

The capsular polysaccharide is a repeating hexasaccharide containing D-glucose, D-galactose, L-fucose, and D-glucuronic acid in a ratio of 1:2:2:1, with a pyruvyl group linked to galactose and an acetyl group linked to fucose (24). When this negatively charged molecule is present in the cell extracts, it may bind specifically to those proteins discussed above (except polypeptide *a*), thereby affecting their isoelectric points and changing the position of these proteins in two-dimensional gels. Since the capsule is extracellular, it is unlikely that its effect on the RNA polymerase and dihydrolipoamide acetyltransferase (cytoplasmic enzymes) in cell extracts has any physiological relevance. However, if one of the unknown affected polypeptides should be identified as a membrane protein, then this information could be important in understanding the attachment of the capsule to the outer membrane. The further importance of the capsule-related effects is to warn investigators using two-dimensional gel electrophoresis about the misleading observations which can occur in studies of encapsulated strains. Even wild-type *E. coli* K-12 ($capR^+$) produces some capsular polysaccharide, and the production is enhanced by growth at low temperature and in minimal medium (24).

Other polypeptide differences between the wild-type and *capR* strains which are not labeled can be seen in Fig. 1. However, these differences are not reproducibly observed.

In conclusion, we found one polypeptide that is either a substrate of the $capR^+$ protease or part of a cascade initiated by $capR^+$ proteolysis. The appearance of this polypeptide is associated with inhibition of cell division, but it is not *recA* dependent, i.e., not part of the SOS response. Instead, it may function in another cellular pathway which affects cell division, the details of which must await further characterization of the protein.

G.W.H. was instructed in the art and science of two-dimensional gel electrophoresis in the laboratory of Frederick C. Neidhardt at the University of Michigan. We express our thanks and appreciation to him and his coworkers, especially Teresa Phillips.

This research was supported by Public Health Service Grant AI 06966 from the National Institute of Allergy and Infectious Disease (to A.M.), American Cancer Society grant MV-69E (to A.M.), and Public Health Service training award GM 07543-04 from the National Institutes of Health (to J.M.S.).

LITERATURE CITED

1. **Adler, H. I., and A. A. Hardigree.** 1964. Analysis of a gene controlling cell division and sensitivity to radiation in *Escherichia coli*. J. Bacteriol. **87**:720–726.
2. **Beck, E., and E. Bremer.** 1980. Nucleotide sequence of the gene *ompA* coding the outer membrane protein II* of *Escherichia coli* K-12. Nucleic Acids Res. **8**:3011–3022.
3. **Berg, P. E., R. Gayda, H. Avni, B. Zehnbauer, and A. Markovitz.** 1976. Cloning of *Escherichia coli* DNA that controls cell division and capsular polysaccharide synthesis. Proc. Natl. Acad. Sci. U.S.A. **73**:697–701.
4. **Bloch, P. L., T. A. Phillips, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 81 *Escherichia coli* proteins. J. Bacteriol. **141**:1409–1420.
5. **Buchanan, C. E., S.-S. Hua, H. Avni, and A. Markovitz.** 1973. Transcriptional control of the galactose operon by the *capR* (*lon*) and *capT* genes. J. Bacteriol. **114**:891–893.
6. **Bukhari, A. I., and D. Zipser.** 1973. Mutants of *Escherichia coli* with a defect in the degradation of nonsense fragments. Nature (London) New Biol. **243**:238–241.
7. **Charette, M. F., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon* (*capR*) protein of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **78**:4728–4732.
8. **Craig, N., and J. W. Roberts.** 1980. *E. coli* recA protein-directed cleavage of phage λ repressor requires polynucleotide. Nature **283**:26–30.
9. **Donch, J., and T. Greenberg.** 1968. Genetic analysis of *lon* mutants of strain K12 of *Escherichia coli*. Mol. Gen. Genet. **103**:105–115.
10. **Gayda, R. C., H. Avni, P. E. Berg, and A. Markovitz.** 1979. Outer membrane protein *a* and other polypeptides regulate capsular polysaccharide synthesis in *E. coli* K-12. Mol. Gen. Genet. **175**:325–332.
11. **Gayda, R. C., and A. Markovitz.** 1978. Altered bacteriophage lambda expression in cell division mutants *capR* (*lon*) of *Escherichia coli* K12. Mol. Gen. Genet. **159**:1–11.
12. **Gayda, R. C., L. T. Yamamoto, and A. Markovitz.** 1976. Second-site mutations in *capR* (*lon*) strains of *Escherichia coli* K-12 that prevent radiation sensitivity and allow bacteriophage lambda to lysogenize. J. Bacteriol. **127**:1208–1216.
13. **Gottesman, S.** 1981. Genetic control of the SOS system in *E. coli*. Cell **23**:1–2.
14. **Gottesman, S., M. Gottesman, J. E. Shaw, and M. L. Pearson.** 1981. Protein degradation in *E. coli*: the *lon* mutation and bacteriophage lambda N and c11 protein stability. Cell **24**:225–233.
15. **Gottesman, S., and D. Zipser.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**:844–851.
16. **Greenblatt, J., and J. Li.** 1981. The *nusA* gene protein of *Escherichia coli*. Its identification and a demonstration that it interacts with the gene N transcription anti-termination protein of bacteriophage lambda. J. Mol. Biol. **147**:11–23.
17. **Gudas, L. J., and D. W. Mount.** 1977. Identification of the *recA* (*tif*) gene product of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5280–5284.
18. **Howard-Flanders, P., E. Simpson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics **49**:237–246.
19. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division mechanism in *E. coli*. Nature **290**:797–799.
20. **Kowit, J. D., and A. L. Goldberg.** 1977. Intermediate steps in the degradation of a specific abnormal protein in *Escherichia coli*. J. Biol. Chem. **252**:8350–8357.
21. **Langley, D., and J. R. Guest.** 1978. Biochemical genetics of the α-keto acid dehydrogenase complexes of *Escherichia coli* K12: genetic characterization and regulatory properties of deletion mutants. J. Gen. Microbiol. **106**:103–117.
22. **Little, J. W., S. D. Edmiston, L. Z. Pacelli, and D. W. Mount.** 1980. Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. U.S.A. **77**:3225–3229.
23. **Mackie, G., and D. B. Wilson.** 1972. Regulation of the *gal* operon of *Escherichia coli* by the *capR* gene. J. Biol.

Chem. 247:2973–2978.
24. **Markovitz, A.** 1977. Genetics and regulation of capsular polysaccharide biosynthesis and radiation sensitivity, p. 415–462. *In* I. W. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press, Inc., New York.
25. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
26. **O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12**:1133–1142.
27. **Phillips, T. A., P. L. Bloch, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 55 additional *Escherichia coli* proteins. J. Bacteriol. **144**:1024–1033.
28. **Roberts, J. W., C. W. Roberts, N. L. Craig, and E. M. Phizicky.** 1978. Activity of the *Escherichia coli recA* gene product. Cold Spring Harbor Symp. Quant. Biol. **43**:917–920.
29. **Schoemaker, J. M., and A. Markovitz.** 1981. Identification of the gene *lon* (*capR*) product as a 94-kilodalton polypeptide by cloning and deletion analysis. J. Bacteriol. **147**:46–56.
30. **Shineberg, B., and D. Zipser.** 1973. The *lon* gene and degradation of β-galactosidase nonsense fragments. J. Bacteriol. **116**:1469–1471.
31. **Takano, T.** 1971. Bacterial mutants defective in plasmid formation: requirement for the lon^+ allele. Proc. Natl. Acad. Sci. U.S.A. **68**:1469–1473.
32. **Walker, J. R., C. L. Ussery, and J. S. Allen.** 1973. Bacterial cell division regulation: lysogenization of conditional cell division lon^- mutants of *Escherichia coli* by bacteriophage lambda. J. Bacteriol. **113**:1326–1332.
33. **Zehnbauer, B. A., E. C. Foley, G. W. Henderson, and A. Markovitz.** 1981. Identification and purification of the lon^+ ($capR^+$) gene product, a DNA-binding protein. Proc. Natl. Acad. Sci. U.S.A. **78**:2043–2047.
34. **Zehnbauer, B. A., and A. Markovitz.** 1980. Cloning of gene *lon* (*capR*) of *Escherichia coli* K-12 and identification of polypeptides specified by the cloned deoxyribonucleic acid fragment. J. Bacteriol. **143**:852–863.